(54) Title: HOMOCONJUGATED IMMUNOGLOBULINS

(57) Abstract

Homoconjugated antibodies with high avidity for antigen have increased therapeutic activies and are utilized in pharmaceutical and diagnostic compositions. The homoconjugates, typically prepared from monoclonal antibodies of the IgG class which bind to the same antigenic determinant, are covalently linked by synthetic cross-linking. The homoconjugates are comprised of at least two immunoglobulin monomers so as to provide an IgG-like molecule which is tetravalent, hexavalent or more for the selected antigen. The homoconjugates are able to cross the placenta. Methods of treatment using these homoconjugates are also provided.

HOMOCONJUGATED IMMUNOGLOBULINS

Related Application

The present application is a continuation-in-part of Ser. No. 07/575,725, filed August 31, 1990.

Background Of The Invention

Monoclonal antibodies offer great promise as exquisitely specific immunotherapeutic agents with potentially minimal side effects. Thus, monoclonal antibodies are being developed for a wide variety of applications, such as the treatment of tumors, infectious diseases and autoimmune disorders, regulation of the immune system, and others. Unfortunately, few monoclonal antibodies have the qualities that enable them to successfully make the transition from research and development to clinical regimen.

The therapeutic or diagnostic usefulness of a monoclonal antibody results from several factors, in addition to simply binding the desired antigen. The antibody must possess sufficient binding affinity, a measure of the inherent strength of the antibody binding to its corresponding epitope. It must also have a relatively high level of avidity, which reflects the overall stability of the antibody-antigen complex and is based on the valency of the antibody (and antigen) and the geometric arrangement of the interacting components. The affinity and avidity of different antibodies can vary widely.

Often the monoclonal antibody which is selected
must be of an appropriate isotype or subclass thereof to
efficiently initiate desired effector functions. These
functions include fixation of complement, binding to
effector macrophages or polymorphonuclear leukocytes, or
other properties that may be required in a particular
therapeutic application. Isotype also affects antibody
bio-distribution, half-life, transplacental passage, and
other characteristics.

In general, IgG antibodies would be preferred
over IgM antibodies for most therapeutic uses. When
compared to IgMs, IgGs typically possess longer in vivo
half-lives, are able to cross the placenta to the fetus,
and when formulated as a pharmaceutical composition may
have a longer shelf life. IgG molecules are monomeric,
however, and have only two antigen binding sites so the
avidity is much lower than with a comparable IgM
antibody, which is pentavalent and has ten antigen
binding sites.

With conventional technology it is frequently
very difficult to identify monoclonal antibodies having
the desired antigen binding specificity, affinity,
avidity and effector functions. Recombinant DNA
techniques have been developed to avoid the unpredictable
and labor intensive method of simply screening large
numbers of antibody-producing fused or transformed cells.
Genes encoding the antigen binding variable (or
hypervariable) regions of an antibody having a desired
binding specificity have been cloned next to genes
encoding antibody constant regions which mediate desired
effector functions. See, for example, U.S. Pat.
4,816,397, European Patent Office publications EP 173,494
and 239,400 and PCT publication WO 89/07142. Such
procedures can also be quite laborious and have had only
limited experimental validation. Even with these
procedures one may still be faced with a recombinant IgG
antibody not having sufficient avidity to initiate

biologically important effector functions, or with IgM
molecules which have a desired therapeutic activity but
suffer from the general disadvantages associated with
IgMs as mentioned above.

The avidity of IgG antibodies could be improved
by increasing the valency of the molecule to greater than
two. More interactions between antibody and antigen
would result in tighter binding and would stabilize the
antibody-antigen interaction, generally an important
attribute for therapeutic use. IgG antibodies of high
avidity (via multivalent attachment) and which have the
desired effector functions would be greatly preferred
over comparable antibodies of low avidity, but to date
antibodies having these characteristics have not been
described.

Accordingly, what is needed in the art is a
means for producing high avidity IgG antibodies having
desired effector functions while avoiding many of the
difficulties inherent in working with IgMs. Quite
remarkably, the present invention fulfills this and other
related needs.

Summary of the Invention

Homoconjugated antibodies possess increased
therapeutic effectiveness when compared to the
corresponding parental antibody monomer. This activity
may be due to, _inter alia_, interactions of higher avidity
and increased effector functions. Accordingly,
antibodies which bind to the same antigen, and more
particularly to the same antigenic determinant, are
covalently bonded via cross-linking to one another by
synthetic chemical coupling to produce such
homoconjugates. Generally, the homoconjugates comprise

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at least two to three antibody molecules, typically of
the IgG class. The antibodies are preferably monoclonal
antibodies, and may be any of a variety of species. For
administration to humans the antibodies will usually be
human or murine in origin or have human constant regions.

Accordingly, pharmaceutical compositions are
provided which comprise a pharmaceutically acceptable
carrier and at least two IgG antibody molecules, which
bind to substantially the same antigenic determinant,
chemically bonded to one another by synthetic covalent
linkage. The homoconjugated antibodies and
pharmaceutical compositions thereof can be used
therapeutically in methods of treatment of antigen
related diseases to, e.g., protect against infection,
such as by _E. coli_ or group B streptococci, inhibit the
growth of tumors, including breast and other tumors,
regulate the immune response, and the like. As
homoconjugates of IgG antibodies are able to pass the
placenta the preparations can be used to treat a fetus in
utero.

In another related aspect the invention
provides a substantial improvement in methods for
therapeutic administration of monoclonal antibodies to a
patient for treatment of an antigen related disease. The
improvement comprises administering to the patient
covalently cross-linked homoconjugated monoclonal
antibodies having at least two IgG antibody molecules
which bind to the same antigenic determinant of the
antigen related to the disease. In preferred embodiments
the antibodies are cross-linked via disulfide bonds.

Brief Description of the Figures

Fig. 1 shows chromatograms of FPLC profiles of the IgG homoconjugate mixtures, with retention time along x-axis and A_{280} along y-axis; Peaks labeled A, B and C, represent trimer, dimer and monomer fractions, respectively;

Fig. 2 illustrates the increased binding activity in EIAs of homoconjugates (dimers or trimers) compared to initial monomers of monoclonal antibody D3, a human IgG monoclonal antibody which binds to the group carbohydrate of group B streptococci;

Fig. 3 illustrates the increased binding activity of homoconjugates (dimers or trimers) compared to initial monomers of monoclonal antibody 5E1-G, a human IgG monoclonal antibody which binds to the capsular carbohydrate of E. coli K1;

Fig. 4 illustrates the increased binding activity in EIAs of homoconjugates (dimers) compared to initial monomers of BR64, a murine IgG monoclonal antibody which binds to a human breast tumor associated antigen;

Fig. 5 illustrates the comparative binding activity of homoconjugated chimeric BR96 antibody against tumor cell lines, where Fig. 5A shows binding activity against human breast tumor cell line H3760B, Fig. 5B shows binding activity against human lung tumor cell line H2707, Fig. 5C shows binding activity against human lung tumor cell line H2987, and Fig. 5D is binding activity against human breast tumor cell line H3396;

Fig. 6 shows the increased opsonic activity against group B streptococci by dimer and trimer homoconjugates of human monoclonal antibody D3 compared to the initial IgG monomer;

Fig. 7 shows the enhanced opsonophagocytosis by monoclonal antibody D3 homoconjugates against group B

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streptococcal strains M94 and I334 compared to the
activity by the D3 monomer antibody;

Fig. 8 shows the increased opsonic activity
against E. coli K1 of dimer and trimer homoconjugates of
human monoclonal antibody 5E1-G compared to the initial
IgG monomer;

Fig. 9 depicts enhanced opsonophagocytosis
conferred by homoconjugates of monoclonal antibody 5E1-G
against strains H16 and A14 of E. coli K1 compared to the
antibody monomer;

Fig. 10 shows increased complement dependent
cytotoxicity against breast tumor cell line H3630 by
dimer homoconjugates of monoclonal antibody BR64 compared
to the initial IgG monomer;

Fig. 11 illustrates the cytotoxicity shown by
BR96 homoconjugates and monomeric monoclonal antibody
against breast tumor cell line H3396; and

Fig. 12 shows the in vivo protection conferred
by homoconjugates of monoclonal antibody D3 and control
monomer at different concentrations of antibody.

Description of the Specific Embodiments

The present invention provides homoconjugates
of monoclonal antibodies against selected antigens, and
methods for preparing such homoconjugates. By chemically
linking antibody molecules, homoconjugates are prepared
which possess increased valency and two or more Fc
regions. By this means a variety of effects may be
accomplished, including, inter alia, increases in binding
avidity, complement fixation, cellular activation,
opsonophagocytosis, etc. Thus the invention provides the
ability to convert antibodies of perhaps limited in vivo
utility to antibodies having characteristics
significantly more conducive to a desired therapeutic

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activity. For example, homoconjugation may serve to convert an IgG monomer of low binding avidity to one of higher avidity and better able to promote effector functions that were perhaps not previously attainable.

By homoconjugate is meant the covalent association or linking of two, three or more antibody molecules which bind to the same antigenic determinant, thereby forming antibody homodimers, homotrimers, etc. The homoconjugates may be prepared from two, three or more different monoclonal antibodies (i.e., those produced by different immortalized cell lines) which bind to the same antigenic determinants (epitopes) on the antigen. The monoclonal antibodies which comprise the homoconjugate may be different (produced by distinct cell lines) but preferably they are the same, i.e., obtained from the same cell line, and thus constitute a relatively homogeneous preparation of monoclonal antibodies with virtually identical antigen binding specificity. By binding to the same or substantially the same epitope is meant to refer to monoclonal antibodies which are capable of reciprocal or non-reciprocal competition with the other for binding to the antigen. One skilled in the art will know how to conduct competition immunoassays, such as by radioimmunoassay or enzyme immunoassay, as generally described in, e.g., U.S. Pat. 3,817,837; Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (1988); and Day, Advanced Immunochemistry, 2d ed., Wiley-Liss Publications, NY (1990), each incorporated herein by reference.

The Fc regions of the monoclonals used for homoconjugation, or other aspects of the immunoglobulin molecule which do not substantially affect antigen binding specificity, may also be altered to produce desired effector functions. For example, it may be desirable to substitute a Fc domain for protein A binding into a molecule not having that capacity, for ease of

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purification or the like. Other substitutions may
provide for decreased immunogenicity, increased or
decreased complement activation, cell receptor binding,
control of catabolic rate, placental and gut transfer,
ability to participate in antibody-dependent cellular
cytotoxicity, and other aspects of immune regulation. A
number of antibody functions have been localized to a
constant region domain or domains. See, Paul,
Fundamental Immunology, Raven Press, New York, NY, 1984,
incorporated herein by reference. A wide variety of
techniques are available to produce recombinant
immunoglobulins, e.g., U.S. Pat. 4,816,397, European
Patent Office publications EP 173,494 and 239,400 and PCT
publication WO 89/07142, each incorporated herein by
reference. Accordingly, the homoconjugated
immunoglobulins may be any of the heavy chains and
subclasses thereof. The light chains may be either kappa
or lambda.

Particularly preferred in the present invention
are homoconjugates of antibodies having gamma heavy
chains, so as to form homoconjugated multivalent IgG
molecules. Within the IgG subclasses of 1, 2, 3 and 4
(human) and 1, 2a, 2b and 3 (murine), human subclasses 1
and 3 and murine subclasses 1, 2a and 2b are generally
preferred for applications requiring maximum complement
fixation, binding to monocytes, macrophages and
polymorphonuclear cells, and the ability to cross the
placenta. The effector functions of human IgG_2 and IgG_4
antibodies may also be substantially increased by the
homoconjugation procedures described herein.

It is also contemplated that under certain
circumstances, depending on the intended use, antibodies
having alpha, mu, epsilon or delta type heavy chains may
also be employed for homoconjugation as described herein.

The binding affinity of the antibodies for use
in homoconjugates will vary, but will generally be at
least 10^{-4} M, typically at least about 10^{-6} M to 10^{-7} M,

and preferably at least about 10^{-8} to 10^{-9} M or greater.
The avidity of the homoconjugates prepared from such
antibodies should generally be at least about 10^{-6} M to
10^{-7} M, and preferably at least about 10^{-8} to 10^{-10} M or
greater. Means for determining affinity and avidity are
known, as described in Day, _Advanced Immunochemistry_,
supra. While the homoconjugates may have quantitative
increases in avidity, generally the homoconjugates should
also have qualitative increases in avidity and effector
functions, e.g., those evidenced by antigen binding tests
and other functional assays as described herein and as
will generally be known to one of ordinary skill in the
art.

The homoconjugated immunoglobulins may be of
any species or combination thereof from which monoclonal
antibodies may be prepared. Although it has generally
been relatively easy to produce murine monoclonal
antibodies of a desired antigen binding specificity, it
has been much more difficult to produce human monoclonal
antibodies of the desired specificity and having the
desired constant region properties. Human monoclonal
antibodies are preferable for many applications,
especially _in vivo_ diagnosis and therapy of humans to
minimize their recognition as foreign by a patient's
immune system.

While murine and human immunoglobulins are most
commonly produced, monoclonal antibodies or portions
thereof originating with other species, such as
lagomorpha, bovine, ovine, equine, porcine, avian or the
like may be employed. It should be understood that the
monoclonal antibody art and genetic engineering
techniques have advanced sufficiently such that antibody
sequences of one species may be interchanged with those
of another species. Thus, as used herein, a "human"
antibody, for example, refers to one that is
substantially human in origin but may also contain some
non-human and/or non-immunoglobulin sequences.

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Similarly, when referring to immunoglobulin, used
synonymously herein with antibody, it will be understood
that some non-immunoglobulin sequences may be present in
the molecule while retaining the ability to bind antigen.

5   Immunoglobulin refers to both whole immunoglobulins and
binding fragments thereof.

The antibodies which are used for
homoconjugation may be substantially monospecific, i.e.,
relatively pure preparations of substantially homogeneous

10   antibodies obtained from polyclonal antisera, or may be
monoclonal antibodies. Monoclonal antibodies which bind
to a desired antigen or epitope thereof are obtained from
an established cell line which secretes them. The
antibody-producing cell line may be isolated from B cells

15   of several species using conventional fusion, viral
transformation or other immortalization techniques well
known to those skilled in the art. For instance, human
monoclonal antibodies may be generated using Epstein-
Barr virus (EBV) transformation, hybridoma fusion

20   techniques, or combinations thereof. See, for example,
Kozbor et al., Proc. Natl. Acad. Sci. USA 79:6651 (1982),
and U.S. Pat. Nos. 4,464,465 and 4,624,921, which are
incorporated herein by reference. By monoclonal antibody
is meant an antibody produced by a clonal, immortalized

25   cell line separate from cells producing antibodies with a
different antigen binding specificity. Thus such
monoclonal antibodies are produced and isolated from
other monoclonal antibodies and, accordingly, in
substantially pure form (relative to other antibodies)

30   and at a concentration generally greater than normally
occurring in sera from the animal species which serves as
the B cell source.

Thus, it should be understood that the
invention is not limited by the antigen binding

35   specificity of the particular homoconjugates exemplified
herein, but rather, it can be used in the treatment of a
variety of antigen related diseases, particularly those

for which monoclonal antibodies have been therapeutically
administered. By antigen related disease is meant a
disease whose manifestation coincide with the clinical
presence of a foreign antigen (e.g., bacteria, virus,
5   tumor or tumor associated antigen) or self antigen (as
with autoimmune diseases). A wide variety of monoclonal
antibodies have been described in the technical and
patent literature, many of which are publicly available
from cell depositories, such as the American Type Culture
10   Collection, 12301 Parklawn Dr., Parkville, MD 20852,
whose catalogue, ATCC Catalogue of Cell Lines and
Hybridomas, 6th ed. (1988), is incorporated herein by
reference. Representative examples of monoclonal
antibodies are described in, e.g., U.S. Pat. Nos.
15   4,596,769, 4,689,299, 4,753,894, 4,834,975, 4,834,976,
4,925,800, and 4,958,009, each of which is incorporated
herein by reference. The methods described herein
provide the ability to produce novel cross-linked
homoconjugates from immunoglobulins obtained from such
20   cell lines.
        The chemically linked homoconjugated
immunoglobulins will be produced by chemical conjugation
of antibodies using well known laboratory procedures,
such as by employing cross-linking reagents. By
25   chemically linked is meant that the immunoglobulin
molecules are synthetically linked, i.e., not produced as
such by a cell, to one another by covalent bonds. A
preferred method of conjugation is the formation of at
least one covalent bond between the immunoglobulin
30   molecules.
        The immunoglobulin molecules are complexed or
chemically bonded together by any of a variety of well
known chemical linking procedures. The Fc regions or Fab
regions may serve as the site of the linkage. The
35   linkage may be direct, which includes linkages containing
a synthetic linking group, or indirect, by which is meant
a link having an intervening moiety, such as a protein or

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peptide, e.g., plasma albumin, or other spacer molecule.
For example, the linkage may be by way of
heterobifunctional or homobifunctional cross-linkers,
e.g., carbodiimide, glutaraldehyde, N-succinimidyl 3-(2-
pyridydithio) propionate (SPDP) and derivatives, bis-
maleimide, 4-(N-maleimidomethyl)cyclohexane-1-carboxylate
(SMCC), cross-linking without exogenous cross-linkers by
means of groups reactive with the individual molecules,
such as carbohydrate, disulfide, carboxyl or amino groups
via oxidation or reduction of the native protein, or
treatment with an enzyme or the like. Methods for
chemically cross-linking antibody molecules are generally
known in the art, and a number of hetero- and
homobifunctional agents are described in, e.g., U.S. Pat.
Nos. 4,355,023, 4,657,853, 4,676,980, 4,925,921, and
4,970,156, and in Harlow and Lane, Antibodies, A
Laboratory Manual, Cold Spring Harbor Press, 1988, Cold
Spring Harbor, NY and ImmunoTechnology Catalogue and
Handbook, Pierce Chemical Co. (1989), each of which
patents and publications is incorporated herein by
reference. In general, such synthetic cross-linking
should not substantially affect the antigen binding
region of the molecules nor the desired effector
functions.

        Detection and purification of the
homoconjugated immunoglobulins may be accomplished by a
variety of techniques, including liquid and affinity
chromatography, gradient centrifugation, and gel
electrophoresis, among others. Increased activity of the
homoconjugates may be measured by quantitative antigen
binding assays, antibody competition experiments,
opsonophagocytic assays, complement dependent
cytotoxicity assays, and the like. These techniques are
familiar to those skilled in the art, and are described
in, for example, Harlow and Lane, supra.

        Homoconjugated antibody preparations with
increased binding ability will likely be useful in the

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treatment and diagnosis of a wide variety of conditions
referred to herein as antigen related diseases. The
homoconjugates will offer significantly improved
therapeutic and diagnostic characteristics compared to
the unconjugated monomeric antibody. Due to the
increased avidity of the homoconjugates, it is now
possible in certain instances to convert a previously
non-protective or weakly protective IgG antibody to be
protective against infection or tumors, for example, or
to act as an immunomodulator by potentiating or otherwise
regulating a host's immune response to a particular
antigen. Where an IgM antibody to an antigen or
particular epitope of the antigen is protective and a
monomeric IgG antibody is non-protective or weakly
protective, a homoconjugate produced using the methods
described herein may provide sufficient avidity to confer
significant protection against infection, cell killing,
etc. For instance, an IgG dimer or trimer homoconjugate
may possess therapeutic anti-infective qualities that may
be found with certain multivalent antibodies such as
IgMs, but also have qualities inherent to IgG monomers,
such as their ability to cross the placenta, to bind to
macrophages and PMNs, and the lack of a requirement for
complement to mediate opsonization. The IgG
homoconjugates may possess other attributes typically
associated with IgGs, such as ease of purification,
increased stability, increased shelf life, and increased
half-life in vivo.

        Although the homoconjugate preparations will be
useful against a range of targets, such as bacterial and
viral antigens, depending of course on the particular
specificity of a homoconjugate's antigen binding region,
they will be especially useful where the killing of
mammalian cells is required. For example, the
homoconjugates can be used for the treatment of cancer
cells which display particular tumor-associated antigens
(e.g., breast or lung tumor associated antigens), the

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inhibition or killing of mammalian cells infected with
viruses or bacteria or cells which express antigens
associated with a particular autoimmune disease. The
homoconjugates can also be used to eliminate selected
5   cells from bone marrow or in the immunosuppression of
graft recipients, etc.

Of course, it is understood that the present
invention is not limited to antibody homoconjugates which
are protective or show other such functional attributes
10   in vivo, as increased avidity also makes feasible an
array of diagnostic procedures perhaps not otherwise
available to a bivalent monomer of low affinity and/or
low avidity.

The ability of the resultant antibodies to
15   inhibit a tumor, such as a breast or lung tumor, to act
as an immunomodulator, or to protect against challenge by
a pathogen, for example, can be measured in a wide
variety of in vitro and in vivo systems, as will be known
to the skilled artisan. An exemplary protocol for
20   protection against E. coli K1, using a homoconjugated
antibody which was non-protective or weakly protective as
an IgG, appears in Example III below.

The novel homoconjugates of monoclonal
antibodies and pharmaceutical compositions prepared
25   therefrom are particularly useful for administration for
prophylactic and/or therapeutic treatment of an antigen-
related disease. Preferably, the pharmaceutical
compositions can be administered parenterally, i.e.,
subcutaneously, intramuscularly or intravenously, or
30   orally. Thus, this invention provides compositions for
parenteral administration which comprise a solution of
the homoconjugated monoclonal antibody preparations or a
cocktail of homoconjugated and monomeric antibodies
dissolved in an acceptable carrier, preferably an aqueous
35   carrier. A variety of aqueous carriers can be used,
e.g., water, buffered water, 0.4% saline, 0.3% glycine
and the like. These compositions may be sterilized by

conventional, well known sterilization techniques. The
compositions may contain pharmaceutically acceptable
auxiliary substances as required to approximate
physiological conditions such as pH adjusting and
buffering agents, toxicity adjusting agents and the like,
for example, sodium acetate, sodium lactate, sodium
chloride, potassium chloride, calcium chloride, etc. The
concentration of antibody in these formulations can vary
widely, i.e., from less than about 0.5%, usually at or at
least about 1% to as much as 15 or 20% by weight and
will be selected primarily by fluid volumes, viscosities,
etc., in accordance with the particular mode of
administration selected, the condition being treated,
e.g., an infectious disease such as a group B
streptococcal or _E. coli_ infection, a tumor, such as
breast carcinoma, etc., and the subject being treated,
i.e., an adult, child or neonate.

Thus, a typical pharmaceutical composition for
intravenous infusion to treat an infection in an adult
could be made up to contain 250 ml of sterile Ringer's
solution, and about 100 mg to 10 grams of antibody.
Actual methods for preparing parenterally or orally
administrable compounds will be known or apparent to
those skilled in the art and are described in more detail
in for example, Remington's Pharmaceutical Science, 16th
ed., Mack Publishing Company, Easton, PA (1982), which is
incorporated herein by reference.

The compositions containing the present
homoconjugated antibodies or a cocktail thereof can be
administered for prophylactic and/or therapeutic
treatments. In therapeutic applications, compositions
are administered to a patient already suffering from a
disease, in an amount sufficient to cure or at least
partially arrest the disease and its complications. An
amount adequate to accomplish this is defined as a
"therapeutically effective dose." Amounts effective for
this use will depend on the severity of the disease,

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i.e., infection, tumor, etc., the age of the patient and
the general state of the patient's immune system.
Generally, the amounts will range from about 0.1 to about
50 mg of antibody per kilogram of body weight per dose,
with dosages of from 5 to 25 mg of antibody per kilogram
per patient being more commonly used. It must be kept in
mind that the materials of the present invention may
generally be employed in serious disease states, that is,
life-threatening or potentially life threatening
situations. In such cases, in view of the minimization
of extraneous substances and the possibility of lower
"foreign substance" rejections which may be achieved by,
e.g., administering allogeneic homoconjugated antibodies
or chimeric homoconjugated antibodies made feasible by
this invention, it is possible and may be felt desirable
by the treating physician to administer substantial
excesses of these antibodies.

In prophylactic applications, compositions
containing the present antibodies or cocktails thereof
are administered to a patient not already in a disease
state to enhance the patient's resistance. Such an
amount is defined to be a "prophylactically effective
dose." In this use, the precise amounts again depend on
the patient's state of health and general level of
immunity, but generally range from 0.1 to 25 mg per
kilogram, especially 0.5 to 2.5 mg per kilogram. A
preferred prophylactic use is for treatment of fetuses
and neonates at risk from infection through their
mothers. When treatment is dependent on passage through
the placenta, the dosage may require adjustment to
reflect the percentage of antibody which is able to pass
from the blood of the pregnant female to that of the
fetus.

Single or multiple administrations of the
compositions can be carried out with dose levels and
pattern being selected by the treating physician. In any
event, the pharmaceutical formulations should provide a

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quantity of homoconjugated antibody sufficient to treat the patient.

The homoconjugated antibodies of the invention may also find several uses _in vitro_. By way of example, the homoconjugated IgG antibodies of Example I below can be used for detecting the presence of group B streptococci or _E. coli_ K1, for vaccine preparation, or the like.

For _in vitro_ diagnostic purposes, the antibodies may be either labeled or unlabeled. Unlabeled homoconjugated antibodies may find particular use in agglutination assays, or they may be used in combination with other labeled antibodies (second antibodies) that are reactive with the homoconjugated antibodies, such as antibodies specific for the Fc regions. Alternatively, the antibody may be directly labeled. A wide variety of labels may be employed, such as radionuclides, particles (e.g. gold, ferritin, magnetic particles, red blood cells), fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are known to those skilled in the art, such as competitive and sandwich assays as described in, e.g., U.S. Pat. 4,376,110, incorporated by reference herein, and Harlow and Lane, supra.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of the presence of a selected antigen. Thus, the subject antibody compositions of the present invention may be provided, usually in lyophilized form in a container, either alone or in conjunction with additional antibodies. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than

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about 5% wt. based on the amount of active antibody, and
usually present in total amount of at least about .0001%
wt., based on the antibody concentration. Frequently it
will be desirable to include an inert extender or
5   excipient to dilute the active ingredients, where the
excipient may be present in from about 1 to 99% of the
total composition. Where a second antibody capable of
binding to the homoconjugated antibodies is employed in
an assay, this will be present in a separate vial. The
10   second antibody is typically conjugated to a label and
formulated in an analogous manner with the antibody
formulations described above.

The following examples are offered by way of
illustration, not by limitation.
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EXAMPLE I
Preparation of Monoclonal Antibody Homoconjugates
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This example demonstrates means for preparing
homoconjugates of several representative monoclonal
antibodies to selected tumor and bacterial antigens. The
homoconjugates were then tested in functional assays
25   described in the examples which follow.

Homoconjugates of the following monoclonal
antibodies were prepared: Monoclonal antibody D3, a
human IgG_1 antibody which binds to the group B
carbohydrate of group B streptococci. 5E1-G, a human IgG_1
30   monoclonal antibody which binds to the capsular
carbohydrate of E. coli K1. BR64, a murine IgG_1
monoclonal antibody which binds to human carcinoma
associated antigen, including colon, breast, ovary and
lung carcinomas. BR64 is on deposit with the American
35   Type Culture Collection, 12301 Parklawn Drive, Rockville,
M.D, as ATCC No. HB 9895. And BR96, also on deposit with
the American Type Culture Collection as ATCC No. HB

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10036, is an IgG human-murine chimeric IgG monoclonal antibody which binds to human lung and breast tumor associated antigens.

Homoconjugates of each of the antibodies were prepared using maleimidobutyrloxysuccinimide and iminothiolane according to the following protocol. Antibodies (1 mg/ml) were dialyzed overnight against a coupling buffer (0.1M Na_2HPO_4-dibasic, seven-hydrate, 0.1M NaCl, pH 7.5). One milliliter of antibody was thiolated with 2-iminothiolane-HCl (Pierce Chemical Co., 50 μl (0.5 mg) of 2-iminothiolane solution (10 mg/ml in coupling buffer) added while mixing. A second aliquot of the antibody (1 ml) was treated with N-γ-maleimidobutyryloxy-succinimide (GMBS)(Calbiochem, La Jolla, CA), 5 μl (14 μg) of GMBS solution (1 mg in 360 μl dimethylformamide (DMF). Each treated aliquot of antibody was incubated 1 hr. at room temp. and then the antibodies were run over PD-10 columns (Pharmacia) pre-equilibrated in coupling buffer. After a void volume of 2.6 ml total, antibodies were collected in double the original volume. The thiolated and GMBS-treated aliquots of antibodies were then mixed and incubated at room temp. for 5 hrs. The reactions were quenched by adding 1 μl of 25mM β-mercaptoethanol (1 μl in 560 μl coupling buffer) and incubating for 15 min. at room temp. The β-ME was stopped by adding 11 μl (11 μg) N-ethylmaleimide (Sigma Chemical Co., St. Louis) made up to 1 mg/ml in DMF. The homoconjugate preparations were dialyzed overnight in phosphate buffered saline (PBS) and separated by size-exclusion chromatography using Superose-6 and Superose-12 FPLC columns (Pharmacia, Uppsala, Sweden). The chromatograms of the FPLC columns for monoclonal antibodies D3, 5E1-G, and BR64 are shown in Fig. 1.

35

EXAMPLE II
Binding Activity of Homoconjugates

5   The ability of the tetravalent and hexavalent
monoclonal antibody homoconjugates to bind antigen was
compared to the binding activity of the bivalent IgG
monomer antibodies. The binding of the anti-GBS
homoconjugates was measured against a GBS strain (I334)
10   bound to microtiter wells using poly-L-lysine (PLL).
Equivalent protein concentrations of untreated antibody
D3 monomer were compared to FPLC fractionated IgG dimer
and trimer homoconjugates. Binding was assayed with
biotin labeled anti-human gamma-chain specific
15   antibodies. The results are shown in Fig. 2, where the
relative binding activities of the dimer or trimer
homoconjugate preparations were significantly greater
than the initial IgG monomer.

          To measure the binding of the anti-E. coli K1
20   antibody homoconjugates, E. coli strain H16 was bound to
microtiter wells using poly-L-lysine. Untreated antibody
5E1-G was compared to homoconjugates of IgG dimer and
trimer, prepared as described above. Equivalent protein
concentrations of antibodies were reacted with the E.
25   coli. Binding was assayed with biotin labeled anti-human
gamma-chain specific antibodies. The results are shown
in Fig. 3, where the relative binding activities of the
dimer and trimer homoconjugate preparations were
significantly greater than the initial IgG monomer.

30   To measure the binding of the anti-breast tumor
antibody, BR64, and homoconjugates thereof, a breast
tumor cell line, 3396, was grown adherently to microtiter
wells. Untreated antibody BR64 was compared to
homoconjugates of IgG dimer and trimer. Equivalent
35   protein concentrations of antibodies were reacted with
the breast tumor cells. Binding was assayed with biotin
labeled anti-murine gamma-chain specific antibodies. The

results are shown in Fig. 4, where the relative binding
activities of the dimer and trimer homoconjugate
preparations were significantly greater than the initial
IgG monomer.

To measure the binding of the anti-breast and
lung tumor antibody, human-mouse chimeric BR96, and
homoconjugates thereof, two breast cell lines (H3396 and
H3760B) and two lung cell lines (H2987 and H2707) were
used as targets. Freshly trypsinized cells were attached
to microtiter plates using PLL and the ELISAs performed
as follows. PLL, made up at 1 µg/ml in PBS, was adsorbed
to Immulon 96-well microtiter plates by incubating 75
µl/well of the PLL solution for 1 hour at room temp.
Carcinoma cell lines (cultured in IMDM with 15% FCS) were
trypsinized, washed twice, and resuspended in PBS at 2 x
10^5 cells/ml. The PLL treated ELISA plates were washed 3
times with saline/Tween (all wash steps done with a
gravity flow wash system). The cell suspension was added
at 100 µl/well (about 20,000 cells/well) and incubated
for 1 hr at 37°C. The plates were then washed 3 times
with saline/Tween. Antibodies were diluted in specimen
diluent (5% nonfat dry milk, 100 µl/L Foam A, 0.01% w/v
thimerosal in PBS) then added to the ELISA plates (100
µl/well) and incubated for 1 hr at room temp. Following
incubation, the plates were washed 3 times with
saline/tween, and peroxidase-conjugated goat anti-human
or mouse IgG (Tago) diluted in specimen diluent was used
as a second step reagent, (100 µl/well) and incubated for
1 hr at room temp. The plates were then washed 5 times
with saline/Tween, and tetramethylbenzidine (TMB)
chromogen (TMB), diluted 1:100 in buffered substrate, was
added (100 µl/well), and plates incubated for 20 minutes.
The reactions were stopped with 100 µl/well of 3N H_2SO_4
and the plates read at dual wavelength, 450/630nm.

Untreated monoclonal antibody BR96 was compared
to homoconjugated BR96 IgG dimers using approximately
equivalent protein concentrations of antibody. The

results, shown in Fig. 5A-D for each of the tested tumor
cell lines, indicate that the relative binding activity
against the four tumor cell lines by the predominantly
dimer homoconjugate preparation was greater than by the
5   initial IgG BR96 monomer.

10   EXAMPLE III
             Increased In Vitro Activity of Homoconjugates

             As an indication of _in vivo_ effectiveness, the
     monoclonal antibody homoconjugates to GBS were tested in
15   an _in vitro_ opsonophagocytic assay. Homoconjugates to _E._
     coli Kl were tested for functional activity in two types
     of opsonization assays described below. Homoconjugates
     of BR64 were tested for _in vitro_ function in a complement
     dependent cytotoxicity assay, and homoconjugates of BR96
20   were tested in a complement independent cytotoxicity
     assay.

     Opsonization of GBS by Homoconjugates of D3
             The opsonophagocytic assays for GBS were
25   performed as follows. Bacteria were prepared by
     inoculating 10 ml of tryptic soy broth (TSB) with 50 μl
     of an overnight broth culture. The tubes were incubated
     at 37°C on a shaker for 3 hours at which time 1.5 ml of
     the culture was centrifuged for 1 min. at 10,000 x g, the
30   spent culture media discarded, and the pellet was
     suspended in 3.5 ml of Hank's balanced salt solution
     containing 0.1% gelatin and 5 mM HEPES (HBSS/Gel). The
     bacterial concentrations were adjusted to about 3 x 10^4
     bacteria/ml by measuring the O.D.$_{600}$ and making the
35   appropriate dilutions (approximately 1:50,000). Human
     neutrophils were isolated according to van Furth and Van
     Zwet ("_In Vitro_ Determination of Phagocytosis and

Intracellular Killing by Polymorphonuclear and
Mononuclear Phagocytes," in Handbook of Experimental
Immunology, Vol. 2, D.M. Weir, ed., 2nd edition,
Blackwell Scientific Publications, Oxford, 36.1-36.24
(1973)) with several modifications. Buffy coat from 5 ml
of heparinized blood diluted 1:2 with PBS was underlayed
with Lymphocyte Separation Medium and centrifuged. The
red blood cell (RBC) pellet was washed once with RPMI
1640 medium and resuspended in an equal volume of 37°C
PBS. Twenty-five ml of this suspension was added to 25
ml of 2% dextran (in 37°C PBS) and the contents gently
but thoroughly mixed end over end. After a 20 min.
incubation at 37°C to allow the RBC's to sediment, the
supernatant (containing neutrophils) was removed, washed
twice in 4°C PBS, once in HBSS/Gel, and suspended in same
to 5×10^7 neutrophils/ml. For the complement source used
with GBS, human serum was thrice adsorbed with live
bacteria (Bjornson, A.B. and Michael, J.G., J. Inf. Dis.,
130 Suppl:S119-S126 (1974)) corresponding to the
organisms used in the assay.

For the assay, into 1.5 ml sterile
polypropylene microfuge tubes were added 250 μl antibody
(test homoconjugates or monomer) preparation in 10% fetal
calf serum in HBSS/gel with HEPES and 100 μl bacterial
suspension (about 3×10^4 bacteria/ml). After 30 minutes
at 37°C, 150 μl containing 75 μl complement, 50 μl
neutrophils (5×10^7 ml), and 25 μl HBSS/gel were added.
The mixtures were incubated on a rotator for 60 minutes
at 37°C, after which they were placed into an ice water
slurry. After 10 minutes, 20 μl from each tube was added
to a 100 mm petri dish containing 3 ml of solidified 0.5%
tryptic soy broth agarose, followed by incubation at
37°C. After 18 hours the colonies were enumerated and
the data was reported as colony forming units (CFU) for
each condition.

The results for homoconjugates of D3 are shown
in Fig. 6, where the dimer and trimer required much less

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antibody, on a nanogram protein basis, to opsonize the
GBS strain tested when compared to the initial IgG
monomer.

     As a further indication of _in vivo_

5 effectiveness, homoconjugates prepared with an additional
monoclonal antibody to the group B carbohydrate of GBS
(D3, produced as generally described in Raff et al., _J._
Infect. Dis. 163:346 (1991) and PCT patent publication WO
91/06305, each of which is incorporated herein by

10 reference) were tested in _in vitro_ opsonophagocytic
assays against two GBS strains, M94 and I334. The
results of the assays are shown in Fig. 7, where it is
evident that the anti-GBS D3 homoconjugates resulted in
increased opsonization of the GBS human clinical

15 isolates. Again, these results suggest that the
homoconjugates will significantly increase the _in vivo_
protective activities of the antibodies when compared to
the parental IgG monomeric monoclonal antibodies.

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Opsonization of E. coli K1 by 5E1-G Homoconjugates

     To isolate human neutrophils, heparinized human
blood (5 ml) was layered onto 3.0 ml of Mono-Poly
Resolving Medium (MPRM, Flow Labs) in polystyrene tubes

25 and centrifuged for 30 minutes at 300 x g at room temp.
After centrifugation, three cell layers were evident,
with the middle layer containing neutrophils. The serum
and top cell layer were removed and discarded, the
neutrophils collected and added to a 50 ml tube

30 containing pre-warmed PBS. The neutrophils were
centrifuged for 10 minutes at 300 x g at room temp., the
supernatant discarded and the cell pellet resuspended
with 10 ml tissue culture media (RPMI-1640) containing
0.5% gelatin, and the cell concentration adjusted to 5 x

35 10^6 cells/ml.

     The assays were performed as follows. To
luminometer tubes (LKB Nuclear) were added 100 μl

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containing appropriate test (5E1-G) or control IgG
monoclonal antibody monomer to P. aeruqinosa flagella,
100 μl log phase growth bacterial suspension (OD_{660}
=0.02), and 100 μl diluted bacteria-adsorbed human serum
complement, final concentration 3.3%. The complement was
thawed just prior to use and received 5 μl of 2 M
$CaCl_2$/ml. The tubes were placed into a prewarmed LKB
Luminometer which allows 24 tubes to be run on a
continuous reading cycle. After 30 minutes in which the
tubes were warmed and periodically mixed, 100 μl of
neutrophils (5 x 10^6/ml) and 600 μl of 10^{-4} M Luminol in
Hank's Balanced Salt Solution were added. Counting
sessions for 25 continuous cycles, which corresponded to
~80 minutes for 24 sample tubes, were initiated. The
chemiluminescence intensity was displayed as millivolts
(mV) with

$$\text{signal:noise} = \frac{\text{mV values for tubes containing the test antibody}}{\text{average of tubes containing negative antibody.}}$$

        The results of the assays are shown in Fig. 8,
where it is evident that the homoconjugates resulted in
increased opsonization of the E. coli organisms than the
initial IgG monomers. The homodimer and homotrimer of
5E1-G were significantly more opsonic than the 5E1-G IgG
monomeric form. As the opsonophagocytic assays are
typically predictive of in vivo ability to protect
animals (see, e.g., U.S. Pat. No. 4,970,070, incorporated
herein by reference), these results suggest that the
dimer and trimer homoconjugates will significantly
increase the in vivo protective activities of the
antibodies when compared to the parental IgG monomeric
antibody.
        As a further confirmation of in vitro efficacy,
and thus in vivo activity, the monoclonal antibody
homoconjugates to E. coli K1 were tested in in vitro
opsonophagocytic assays, as described above, against two

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additional E. coli K1 strains, H16 and A14. As shown in
Fig. 9, the anti-E. coli K1 homoconjugates resulted in
increased opsonization of the human clinical isolates,
suggesting that the predominantly dimer homoconjugate
preparations will significantly increase the in vivo
protective activities of anti-E. coli K1 monoclonal
antibodies.

Complement Dependent Cytotoxicity BR64 Homoconjugates

In vitro functional assays were also used to
demonstrate the increased functional activity of the
anti-tumor antigen homoconjugated monoclonal antibodies.
For testing BR64 homoconjugates, target tumor cells
(H3630) were labeled with ^{51}Cr by incubation 1 x 10^{6}
cells/0.3 ml tissue culture media in 100 μCi of ^{51}Cr for
1 hour at 37°C, 6% CO_2. After washing to remove excess
^{51}Cr, 2 x 10^{4} labeled cells in 67 μl media (RPMI-1640
plus 15% fetal bovine serum) were added per microtiter
plate well. Next, 67 μl of the appropriately diluted
test monomer (BR64), a negative control monomer (Mab
96.5), or homoconjugated (dimer) monoclonal antibody was
added to duplicate wells. Finally, 67 μl of freshly
thawed human serum complement was added to each well, the
plates covered with parafilm and incubated at 37°C for 4
hours. After incubation, plates were centrifuged at 400
g for 10 minutes, and 100 μl of supernatant was removed
from each well and placed in 12 x 75 mm polystyrene
tubes. The tubes were counted in a gamma counter. The
following controls were included in each assay:

Table I

Well	Media	Serum	Diluted Antibody	Target Cell
Spontaneous Release	134§	–	–	67
Complement Toxicity	67	67	–	67
Total Incorporation	134	–	–	67
Maximum Release*	67	–	–	67
Antibody Alone	67	–	67	67

§Amounts are expressed as µl/well.

*Prior to incubation of the assay, these are the only wells which contain less than 201 µl, because the wells later receive 67 µl Triton X-100 to lyse labelled target cells.

The percentage kill (% kill) was calculated from the following formula:

$$\frac{[\text{Test (mean CPM)} - \text{Hc' control (mean CPM)}]}{\text{Total incorporation [mean CPM} - \text{Hc' control (mean CPM)}]} \times 100 = \% \text{ kill}$$

where CPM is counts per minute as average of duplicate samples obtained from measurement in gamma counter and Hc' is Complement Toxicity control.

The results of the assays are shown in Fig. 10, where it is evident that the homoconjugated BR64 resulted in eight times greater killing of the targeted tumor cells than the initial IgG BR64 monomer. The CDC assay is generally predictive of *in vivo* ability to protect animals against tumors. These results suggest that the homoconjugates will significantly increase the utility of such antibodies *in vivo* against tumors, particularly when compared to parental IgG monomer antibodies.

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Increased Complement Independent Cytotoxicity of Chimeric BR96 Homoconjugates

Target tumor cells (H3396) at 5×10^5 cells/tube
5   were mixed with 100 μl of test antibody and were
incubated at 37°C for 30 minutes. Cells were pelleted
and mixed with the appropriate concentration of propidium
iodide (Sigma, 10 μg/tube). Propidium iodide is a DNA
reactive stain that only penetrates the membrane of dead
10   or dying cells. Therefore, by quantitating the number of
fluorescent cells within the population, the number of
dead cells can be determined (Hellstrom et al., Cancer
Res., 50:2183-2190 (1990)). After incubation for 10
minutes, the cells were washed in tissue culture media
15   containing 15% fetal calf serum, resuspended in same, and
placed on ice. The cells were analyzed for fluorescence
on an EPICS Fluorescence Activated Cell Sorter which
quantitates live and dead cells on the basis of
fluorescence and size (small and large represent dead and
20   live cells, respectively). The results (Fig. 11) showed
that the BR96 homoconjugate dimers were dramatically more
effective in killing the tumor cells than the initial
monomer. These results suggest that the homoconjugates
will significantly increase the utility of such
25   antibodies in vivo against tumors.

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EXAMPLE IV

In Vivo Protection Against E. coli K1 Infection in
Neonatal Rats Using IgG Homoconjugates

Outbred Sprague-Dawley rat pups less than 48
hours old (housed with their mothers) were injected
intraperitoneally with approximately 72 E. coli K1
organisms, and 2 hours later received 1 or 5 µg of dimer
homoconjugates of 5E1-G, or 100 µg of monomeric 5E1-G
antibody, or control IgG and IgM antibodies. In all
experiments, the rat pups were examined daily for
symptoms and were scored for survival. The results of
the experiments, shown in Table II below, demonstrate
that 5 µg of the dimer homoconjugates of 5E1-G antibody
protected significantly more animals from death when
compared to animals receiving twenty times the amount
(100 µg) of monomeric antibody.

Table II
Protection by Homoconjugates Against
E. Coli K1 Infections

Antibody (per rat)	Dose (per rat)	n (rats/group)	%Survival (Survivors/Challenged)	p value
5E1-IgM	20 ng	26	100%	<0.001[*]
5E1-IgG Monomer	100 µg	15	40%	<0.01
5E1-IgG Conjugate	5 µg	14	78%	<0.01
5E1-IgG Conjugate	1 µg	14	29%	<0.05
21B8 (Negative control)	100 µg	24	0	
No antibody control	-	25	0	

[*]Based on survival in experimental group versus
survival in negative control and controls receiving no
antibody.

EXAMPLE V

Transplacental Passage of Homoconjugated
Antibody to Fetuses of Pregnant Rats

The ability of the homoconjugated IgG antibody
to pass through the placenta and into the fetus, and thus
into the subsequently delivered offspring, was compared
with the monomeric antibody. An infant rat model was
used as an animal model. Similar rat models have been
used to predict the transplacental passage of antibody
and other molecules to human fetuses. See generally,
Brambell, Frontiers Biol. 18:234-276 (1970).

Two to three days prior to their anticipated
delivery date, pregnant rats were injected intravenously
with 40 μg of either monomeric 5E1-G IgG (monomer) (Dams
1 and 2) or homoconjugated dimeric IgG (Dams 3 and 4).
Blood samples were collected from the dams two hours
after antibody administration and on the day of delivery,
and from the neonatal rats just after birth. Total human
IgG and human IgG anti-E. coli K1 antibody were
determined in each blood sample using individually
designed quantitative binding assays (ELISA's). By using
anti-human IgG-specific enzyme-labeled secondary
antibodies, rat IgG was neither detected nor interfered
with the quantitation of the injected human IgG.

The amount of transplacentally passaged
antibody was determined as follows. Anti-human gamma
chain antibody was attached to microtiter plates using
carbonate buffer. After adding diluted serum samples
from the dams or pups, binding was assayed with biotin
labeled anti-human gamma chain-specific antibodies.
Since one group of dams received only conjugated
antibody, any human IgG detected in pup sera should be
transplacentally passed homoconjugate.

The experiments showed that the monomeric and
homoconjugated IgG antibodies were transplacentally

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passed with approximately equal efficiency. Therefore, the homoconjugated IgG monoclonal antibody should be useful when administered prophylactically to pregnant females at risk of having a neonate with an increased likelihood of developing a life-threatening infection, such as by E. coli K1 in the case of the present embodiment. The data also support the use of these homoconjugates in transplacental treatment of a variety of other infections and tumors.

EXAMPLE VI

Transplacental Passage of Homoconjugated Monoclonal Antibody to Group B Streptococci

This Example demonstrates the transplacental passage of homoconjugated monoclonal antibody D3.

The experiments were performed as generally described in Example V for the homoconjugated monoclonal antibody to E. coli K1.

The results, shown in Table III, below, indicated that both the monomeric and homoconjugated IgG antibodies were transplacentally passed.

TABLE III: Transplacental Passage of Homoconjugated Antibody From Pregnant Rats to Their Neonates

| Source | Time Post Injection | Antibody Injected | |
		Homoconjugate	Monomer
Dams	2 Hours	1.45 ± 0.4[a]	2.4 ± 0.3
Dams	3 Days (Day of Delivery)	0.14 ± 0.04	0.11 ± 0.03
Pups	Day of Delivery	0.41 ± 0.1	0.70 ± 0.2

[a] Concentration of human IgG in rat serum (μg/ml)

Accordingly, the homoconjugated IgG monoclonal
antibody is useful administered prophylactically or
therapeutically to pregnant females likely to deliver a
5   neonate susceptible to developing or already having an
infection, such as by group B streptococci or E. coli K1.
The present invention also makes possible the use of the
homoconjugates in transplacental treatment of a variety
of other infections and tumors.

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15   EXAMPLE VII

In Vivo Protection Against Group B Streptococcal
Infection With IgG Homoconjugates

This Example describes the use of
20   homoconjugates of the D3 monoclonal antibody to protect
against group B streptococcal infection in vivo,
consistent with and confirming the results of the in
vitro opsonophagocytic assays.

As generally described for the E. coli K1 protection
25   studies described in Example IV above, outbred Sprague-
Dawley rat pups less than 48 hours old (housed with their
mothers) were injected intraperitoneally with
approximately 100 GBS organisms two hours after receiving
an intraperitoneal injection of either 20, 4, 0.8 or 0.2
30   μg of predominantly dimer homoconjugate preparations, 80,
20, or 4 μg of monomeric D3, or control IgG. In the
experiments, rat pups were examined daily for seven days
and were scored for symptoms and survival. The results
from two experiments (data pooled, 25 animals/group),
35   shown in Fig. 12, demonstrate the increased in vivo
protective activity against GBS of the dimer
homoconjugates of human monoclonal antibody D3 compared

to the initial IgG monomer. As little as 4 μg of
homoconjugated dimer protected animals nearly as well as
.that conferred by 80 μg of monomer.

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        Although the present invention has been
described in some detail by way of illustration and
example for purposes of clarity and understanding, it
will be apparent that certain changes and modifications
10 may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1.   A pharmaceutical composition which
comprises covalently cross-linked homoconjugated
monoclonal antibodies having at least two IgG antibody
molecules which bind to the same antigenic determinant,
and a pharmaceutically acceptable carrier.

2.   The pharmaceutical composition of claim 1,
the homoconjugated monoclonal antibodies having two
antibody molecules.

3.   The pharmaceutical composition of claim 1,
the homoconjugated monoclonal antibodies having three
antibody molecules.

4. The pharmaceutical composition of claim 1,
wherein the antibodies are cross-linked by disulfide
bonds.

5.   The pharmaceutical composition of claim 1,
wherein the antibodies are human.

6.   The pharmaceutical composition of claim 1,
wherein the antibody molecules are murine.

7.   The pharmaceutical composition of claim 1,
wherein the antibody molecules are murine-human
chimerics.

8.   The pharmaceutical composition of claim 5,
wherein the human antibody heavy chain is an IgG_1.

9.   The pharmaceutical composition of claim 1,
which is protective against infection due to E. coli K1.

10.   The pharmaceutical composition of claim 1, which is protective against infection due to group B streptococci.

11.   The pharmaceutical composition of claim 1, wherein the homoconjugated monoclonal antibodies bind to a tumor associated antigen and inhibit growth of breast tumor cells.

12.   The pharmaceutical composition of claim 1, wherein the homoconjugated monoclonal antibodies are capable of crossing the placenta.

13.   The pharmaceutical composition of claim 1, wherein the constant regions of the light and heavy chains of the antibody molecules are human.

14.   The pharmaceutical composition of claim 1, wherein the cross-linked antibodies are derived from the same cell line.

15.   A method of treating a patient having a disease related to an antigen, the method comprising administering to the patient a therapeutically effective amount of homoconjugated monoclonal antibodies including at least two covalently cross-linked IgG antibody molecules which bind to the same determinant of the antigen.

16.   The method of claim 15, wherein the antigen related disease is group B streptococcal infection.

17.   The method of claim 15, wherein the antigen related disease is E. coli K1 infection.

18.   The method according to claim 15, wherein
the homoconjugated monoclonal antibodies are administered
to a pregnant patient and the homoconjugates are able to
pass through the placenta into fetal circulation.

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19.   The method according to claim 18, wherein
the homoconjugated monoclonal antibodies are able to
treat the fetus for the antigen related disease.

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20.   The method of claim 19, wherein the
antigen related disease is infection by group B
streptococci or E. coli K1.

21.   The method of claim 15, wherein the

15   antigen related disease is breast tumor and the
homoconjugated monoclonal antibodies bind to a breast
tumor associated antigen.

22.   In a method for therapeutic administration

20   of monoclonal antibodies to a patient for treatment of a
disease related to an antigen, the improvement which
comprises administering to the patient homoconjugated
monoclonal antibodies including at least two covalently
cross-linked IgG antibody molecules which bind to the

25   same antigenic determinant of the antigen.

Chromatograms of
Homoconjugate Mixtures

D3 (Anti-GBS)

5E1-G
(Anti-E. coli K1)
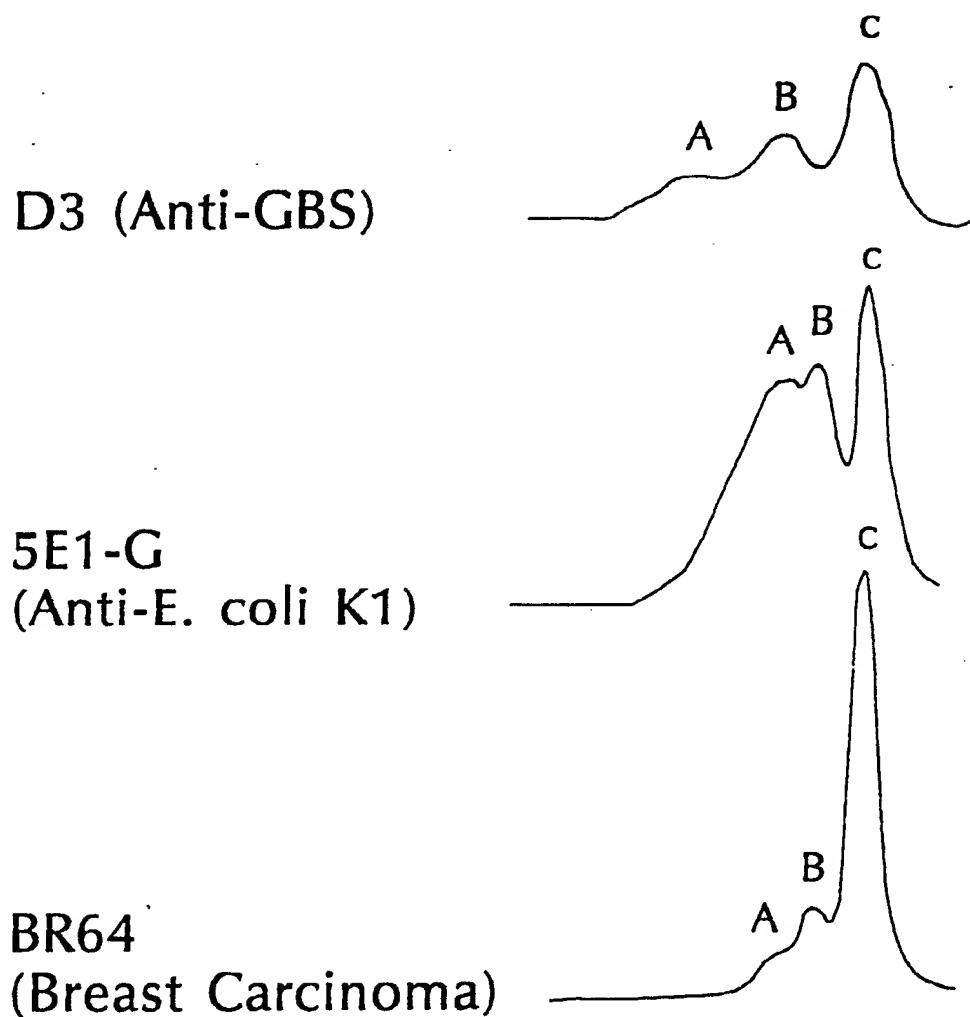
BR64
(Breast Carcinoma)
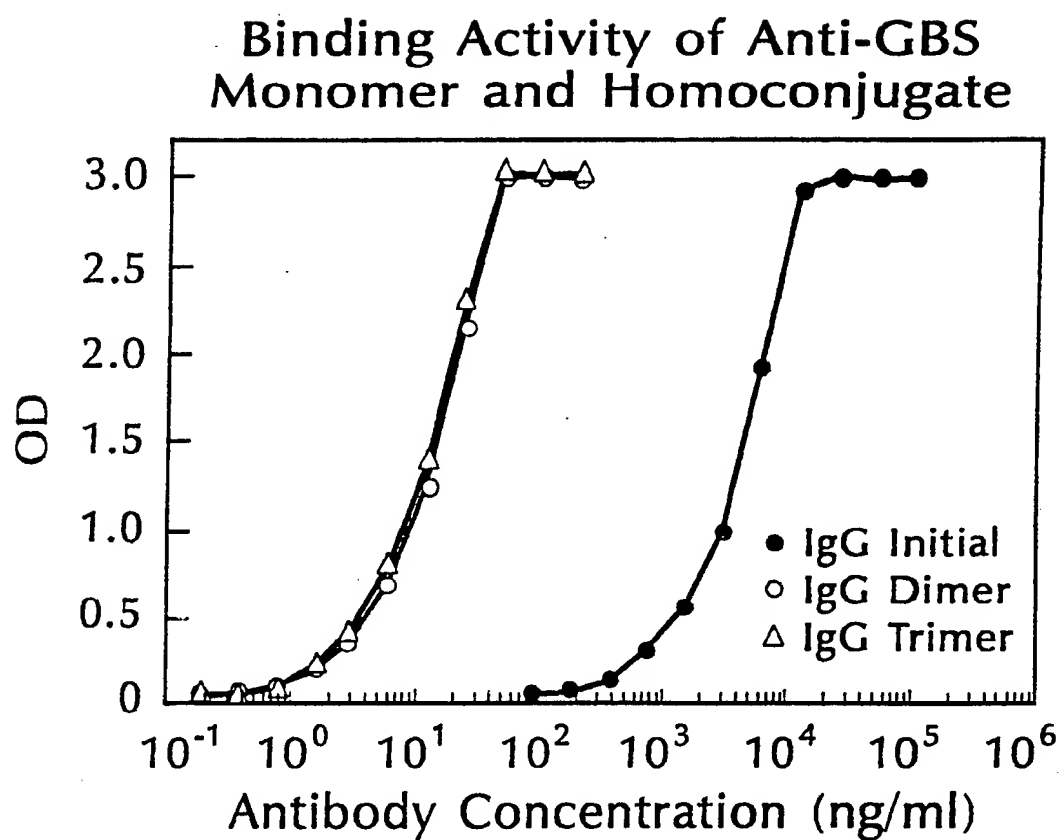
Figure 1

Figure 2

Binding Activity of Anti-E. coli K1 Monomer and Homoconjugate
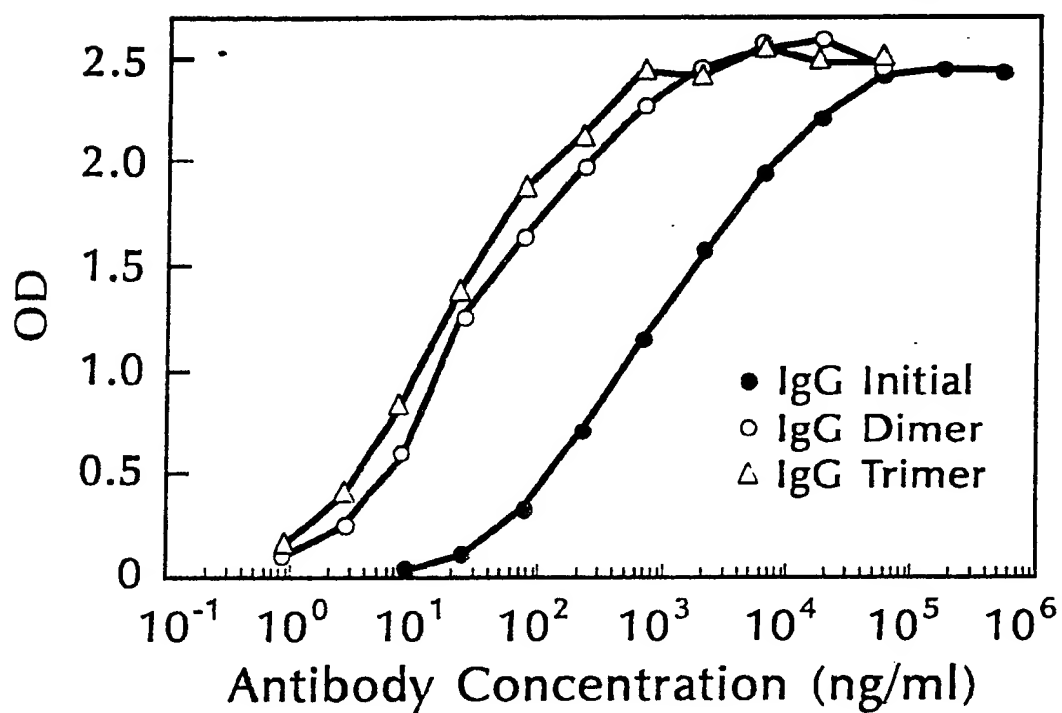
Figure 3

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Binding Activity of Murine Anti-Tumor (BR64) Monomer and Homoconjugate
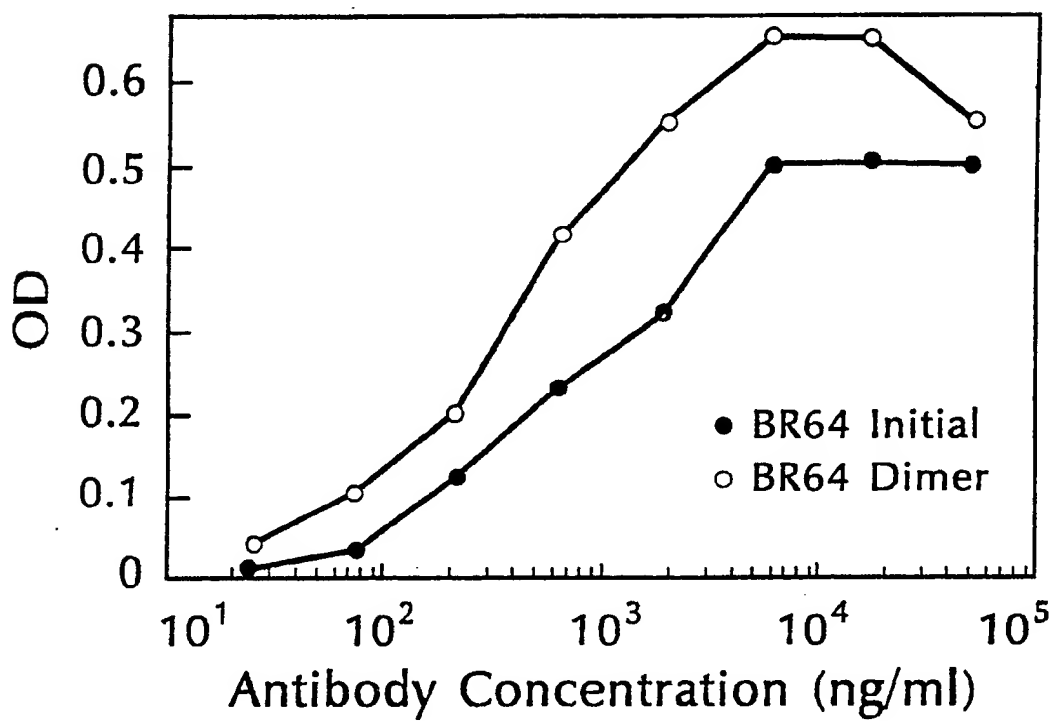
Figure 4

ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H3760B Cells
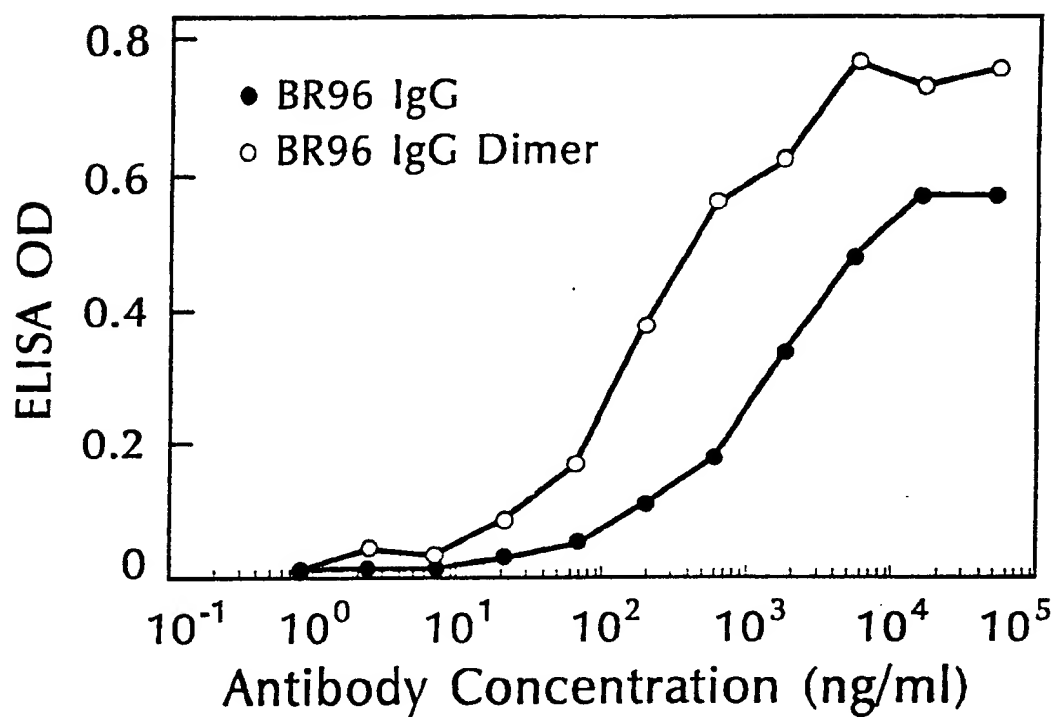
Figure 5A

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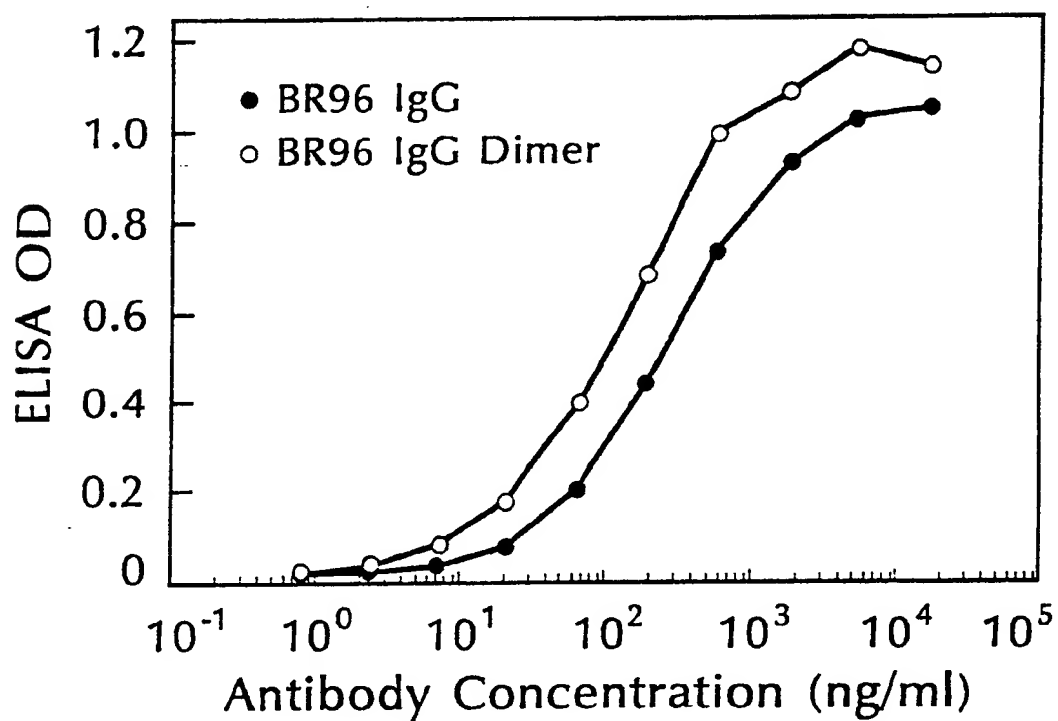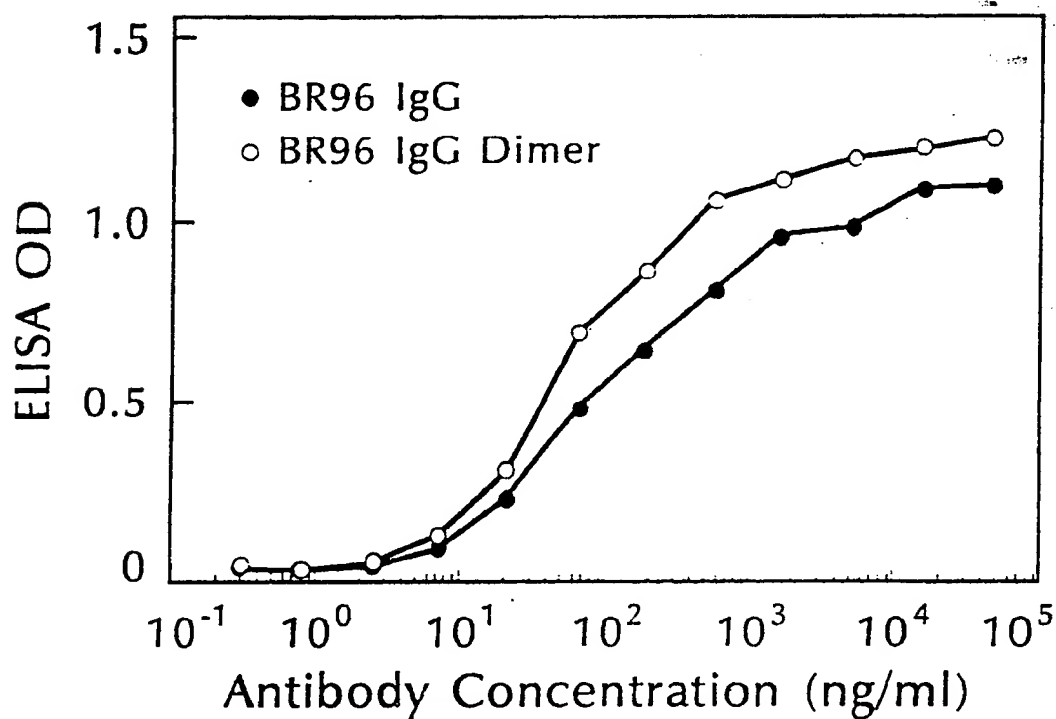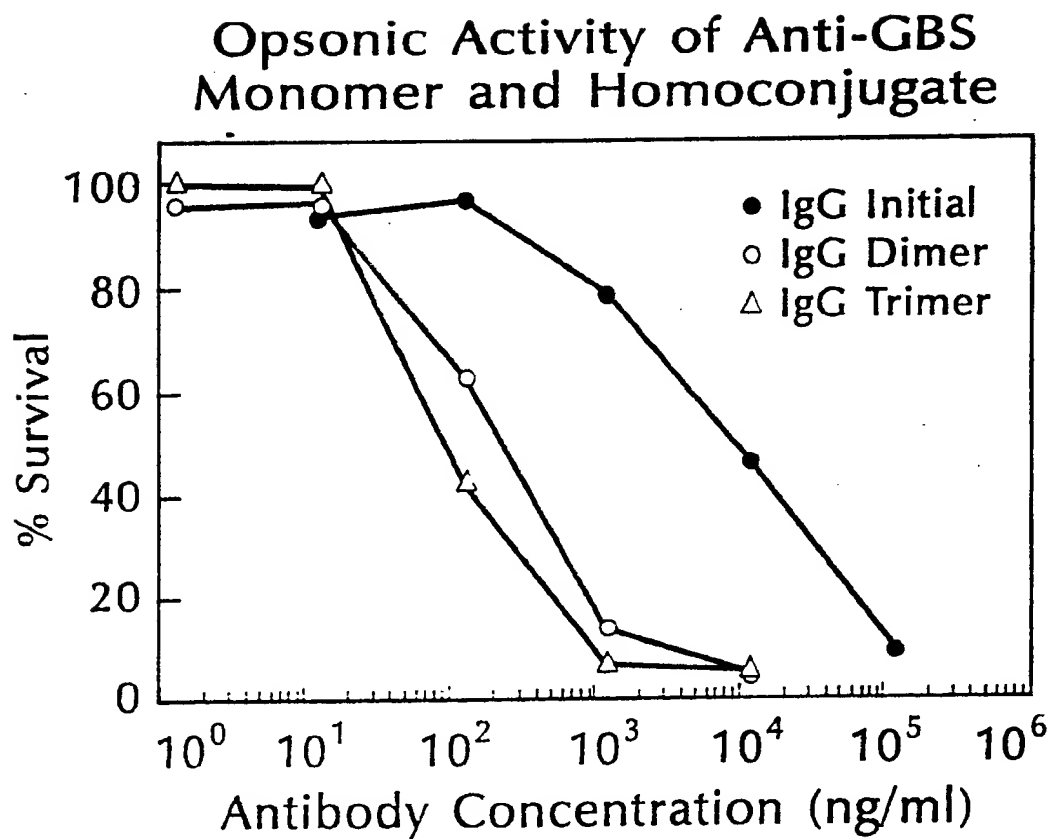## ELISA Binding Activity of BR96 Homoconjugate on PLL-Fixed H2707 Cells
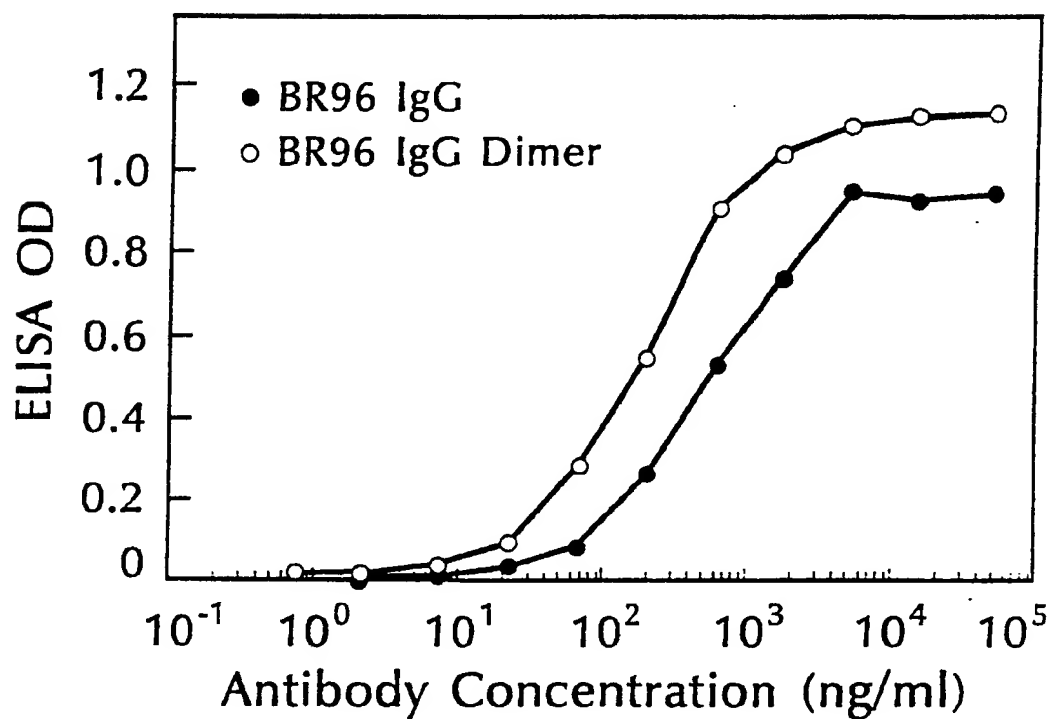
Figure 5B

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ELISA Binding Activity of BR96 Homoconjugate
on PLL-Fixed H2987 Cells

Figure 5C

ELISA Binding Activity of BR96 Homoconjugate
on PLL-Fixed H3396 Cells

Figure 5D

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Opsonic Activity of Anti-GBS Monomer and Homoconjugate

Figure 6

Opsonophagocytic Assay:
D3 IgG Homoconjugate(HC) vs.
Group B Strep Strains M94 and I334
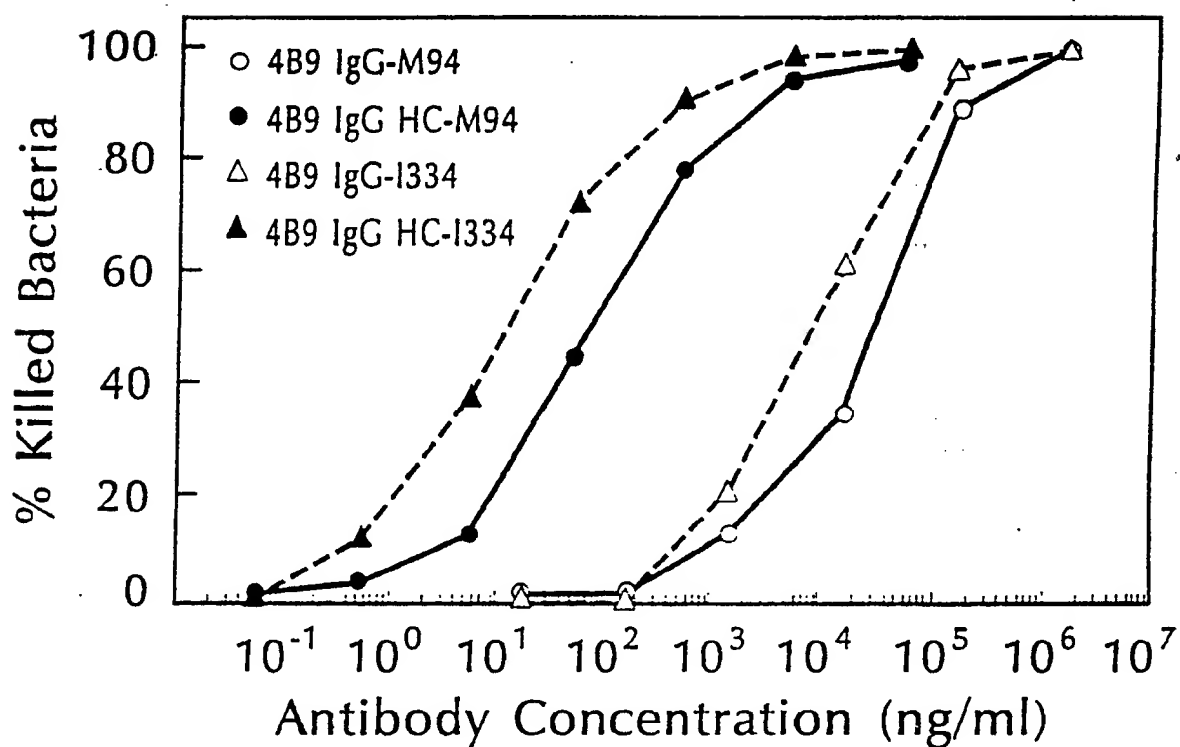
Figure 7
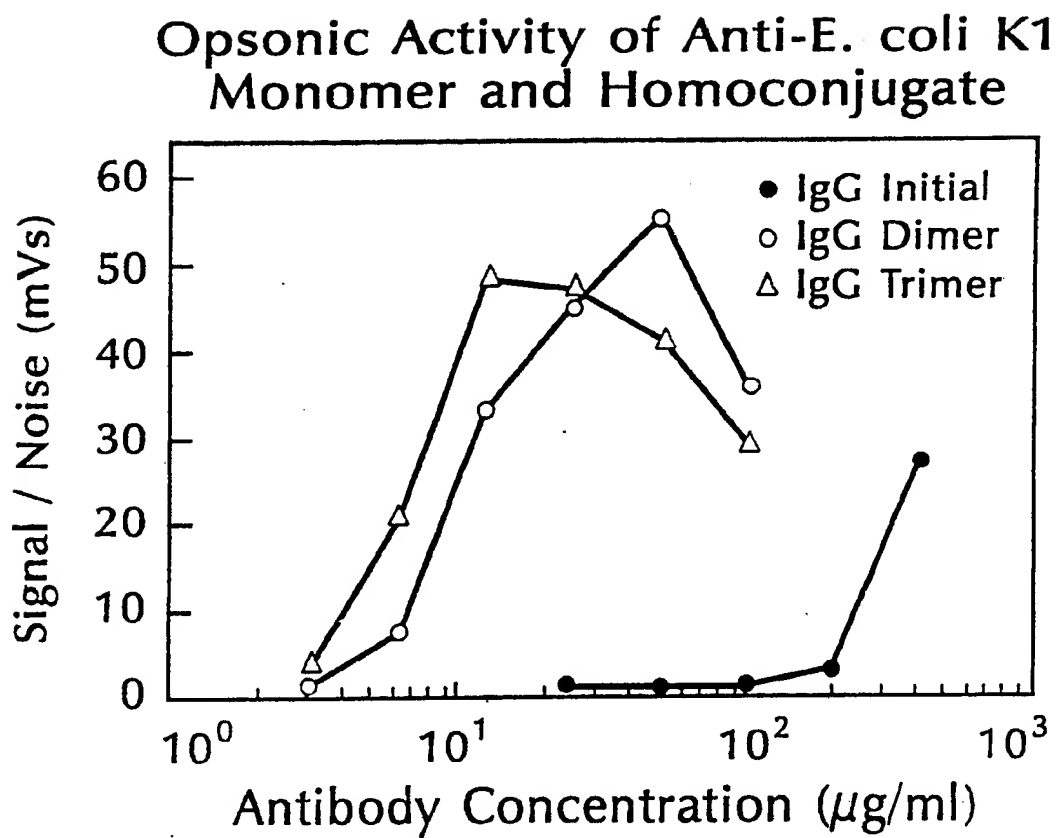
Figure 8

Opsonophagocytic Assay: 5E1 IgG Homoconjugate
vs. E. coli K1 Strains H16 and A14
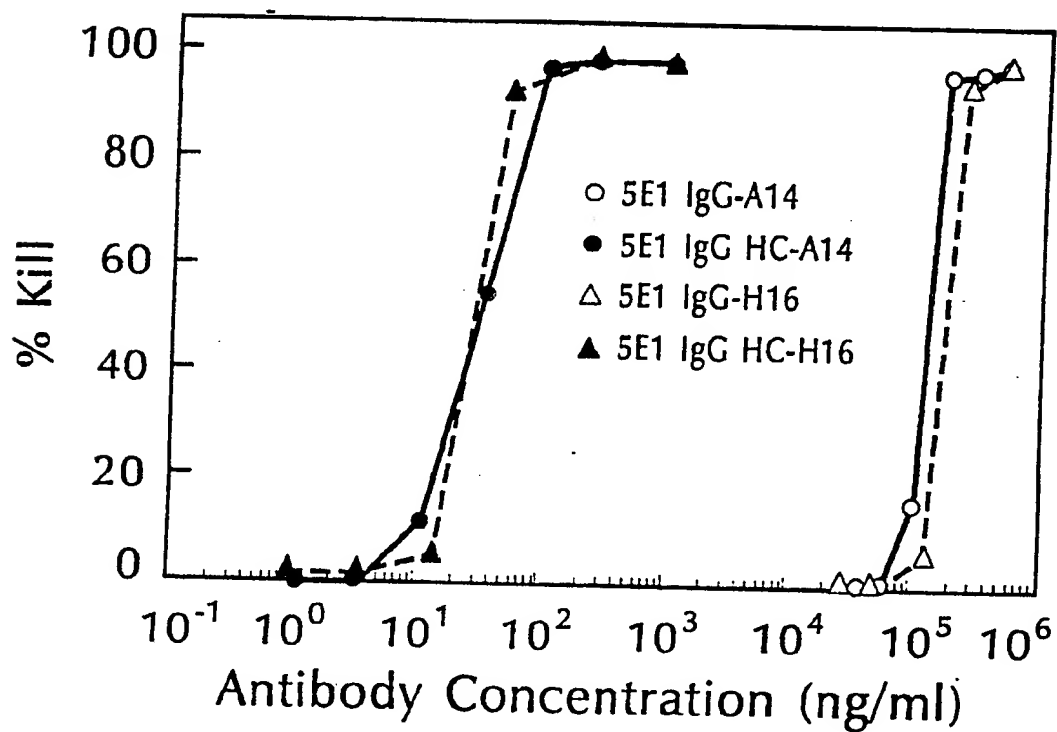
Figure 9

CDC Assay with BR64 Homoconjugate Using Breast Carcinoma Cell Line H3630
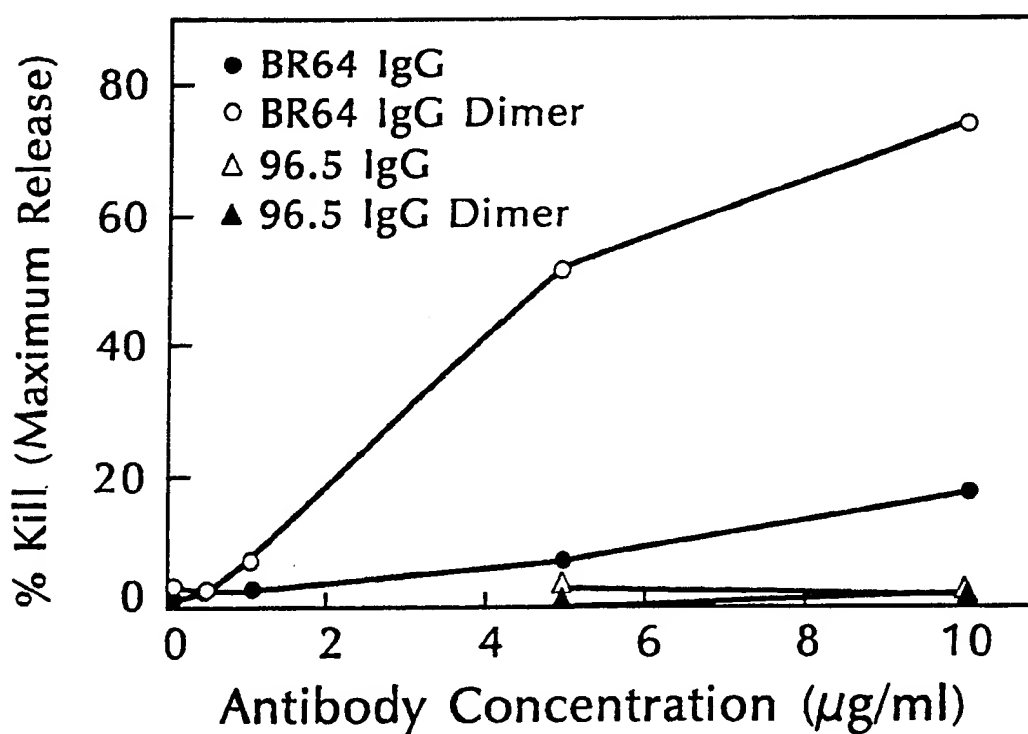
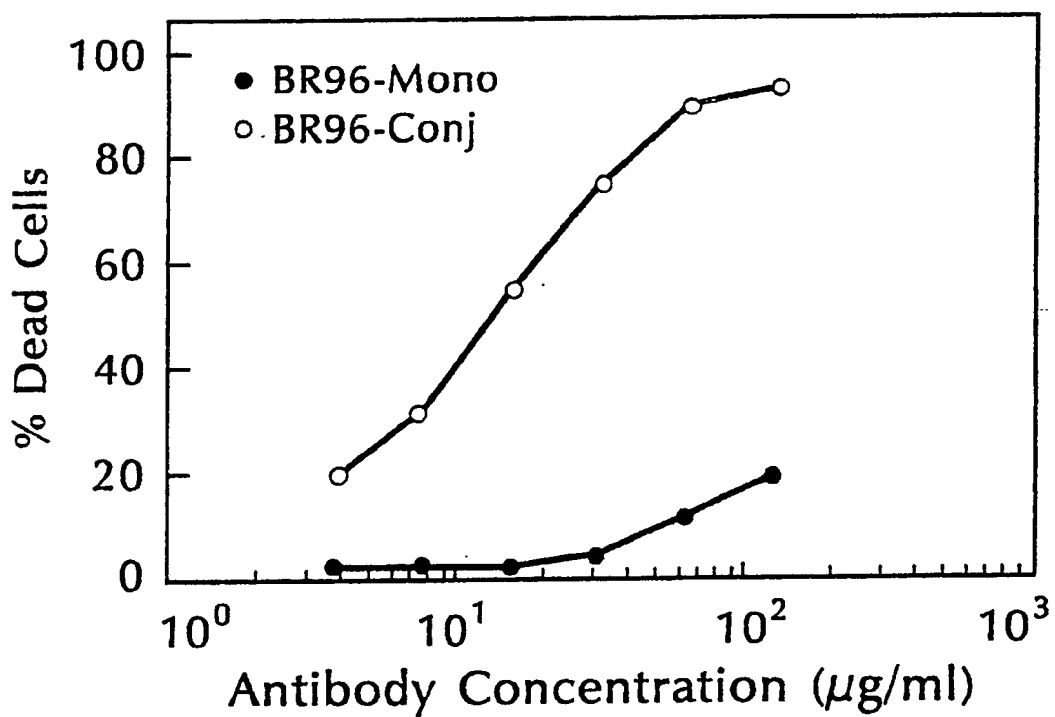Figure 10

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Cytotoxicity by BR96 Against
Cell Line H3396

Figure 11

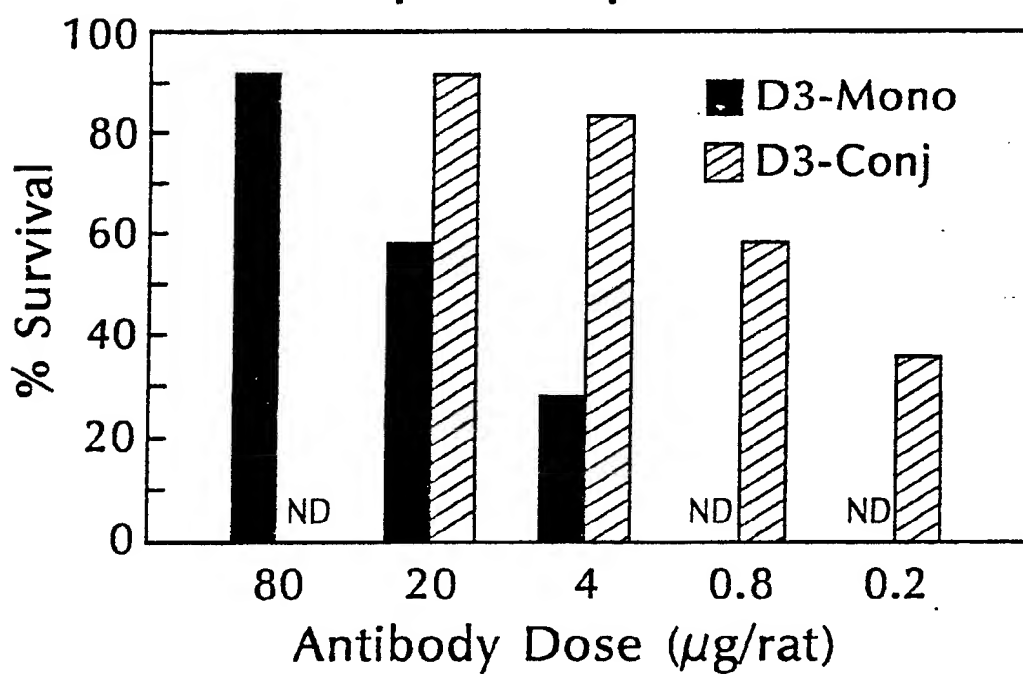## Protection by Homoconjugate Against Group B Strep Infections

Figure 12

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/395
U.S. CL.: 424/85.8

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	424/85.8; 530/387; 435/240.27

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

DATABASES: **DIALOG** (Files 5, 155, 73, 72, 76, 159, 399, 440, 144, 34)
USPTO AUTOMATED PATENT SYSTEM (File USPAT; 1971-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	J. EXP. MED., Volume 168, issued September 1988, RAFF, H.V., ET AL., "Human Monoclonal Antibodies To Group B Streptococcus", pages 905-917, see the entire document.	1-22
Y	J. CHROMATOGRAPHY, Volume 489, Issued 1989, BUSH, D.A. ET AL., "ISOELECTRIC FOCUSING OF CROSS-LINKED MONOCLONAL ANTIBODIES", pages 303-311, see the entire document.	1-14
Y	V. GHETIE, ET AL., METHODS IN ENZYMOLOGY, Volume 92, issued 1983, pages 523-543, "Preparation and Applications of Multivalent Antibodies with Dual Specificity", see the entire document.	1-14
Y,P	SCIENCE, Volume 252, issued 03 May 1991, SHUFORD, W. ET AL., "Effect of Light Chain Oligomerization and in vivo Efficacy", pages 724-727, see the entire document.	1-22

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 October 1991	0 4 NOV 1991
International Searching Authority	Signature of Authorized Officer
ISA/US	Susan L. Futrovsky (vsh)